REMARKS

Applicants respectfully request the Examiner to reconsider the present application in view of the foregoing amendments to the claims and the following remarks.

Status of the Claims

Upon entry of the present Amendment, claims 2-15 are currently pending in the present application. Claims 2, 3, 5, 11, and 13-15 have been amended without prejudice or disclaimer of the subject matter contained therein. No new matter has been added by way of the amendments. The amended claims further define and clarify the invention. Thus, no new matter has been added.

Based upon the above considerations, entry of the present Amendment is respectfully requested.

37 C.F.R. § 1.132 Declaration

An executed 37 C.F.R. § 1.132 Declaration of Dr. Ichiro Hirao is enclosed with the instant Amendment.

The Examiner is respectfully requested to review Dr. Hirao's enclosed Declaration at this time, as it is material to a consideration of whether the below cited references renders obvious instantly pending claims 2-9 and 11-14.

Issue Under 35 U.S.C. § 103(a), Obviousness

Claims 2-9 and 11-14 stand rejected under 35 U.S.C. § 103(a) as unpatentable over

Froehler *et al.*, U.S. Patent No. 6,447,998, U.S. Patent No. 6,495,672 or US Patent Publication No. 2003/0120065 (hereinafter "Froehler *et al.*"), in view of Ohtsuki *et al.*, *"Unnatural Base Pairs for Specific Transcription,"* Proc. Natl. Acad. Sci., Vol. 98, (2001), pages 4922-4925 (hereinafter "Ohtsuki *et al.*") and Guo *et al.*, *"Inhibition of DNA Polymerase Reactions by Pyrimidine Nucleotide Analogues Lacking the 2-Keto Group,"* Nucleic Acids Research, 1998, Vol.26, No.8. p.1863-1869 (hereinafter "Guo *et al.*"). See the Office Action dated July 11, 2008 at pages 2-5 (hereinafter "Office Action"). Applicants respectfully traverse.

Although Applicants disagree, in order to advance prosecution, claims 2, 3, 5, 11, and 13-15 have been amended, without prejudice or disclaimer, to further clarify and define the invention.

Graham v. John Deere, 383 U.S. 1, 17, 148 USPQ 459, 467 (1966), has provided the controlling framework for an obviousness analysis. A proper analysis under § 103(a) requires consideration of the four *Graham* factors of: determining the scope and content of the prior art; ascertaining the differences between the prior art and the claims that are at issue; resolving the level of ordinary skill in the pertinent art; and evaluating any evidence of secondary considerations (e.g., commercial success; unexpected results). 383 U.S. at 17, 148 USPQ at 467.

M.P.E.P. § 2143 sets forth the guidelines in determining obviousness. But before the Examiner can utilize these guidelines, the Examiner has to take into account the factual inquiries set forth in *Graham v. John Deere*; *supra*. To reject a claim based on the above mentioned guidelines, the Examiner must resolve the *Graham* factual inquiries. MPEP §2143.

If the Examiner resolves the *Graham* factual inquiries, then the Examiner has to provide some rationale for determining obviousness, wherein M.P.E.P. § 2143 sets forth the rationales that were established in *KSR Int'l Co. v Teleflex Inc.*, 82 USPQ2d 1385 (U.S. 2007).

Applicants respectfully submit that the Examiner has not appropriately resolved the *Graham* factors, including the factors of determining the scope and content of the prior art and ascertaining the differences between the prior art and the claims that are at issue. Based on the following, Applicants maintain that the above mentioned *Graham* factors actually reside in Applicants' favor. Additionally, Applicants submit that since the Examiner did not resolve the *Graham* factors, the rationale the Examiner provides for combining the cited references is improper.

Applicants respectfully submit that the presently claimed invention is distinct from and unobvious over Froehler *et al.* combined with Ohtsuki *et al.* and Guo *et al.*

The instant invention

The present invention relates to providing a nucleoside or nucleotide having a 5-substituted-2-oxo(1H)-pyridin-3-yl group as a base. In the nucleoside or nucleotide of the present invention, the 5-position of the above base is preferably substituted with a substituent selected from the group consisting of the following:

1) a photoreactive group selected from iodine and bromine;

2) biotin or a derivative thereof;

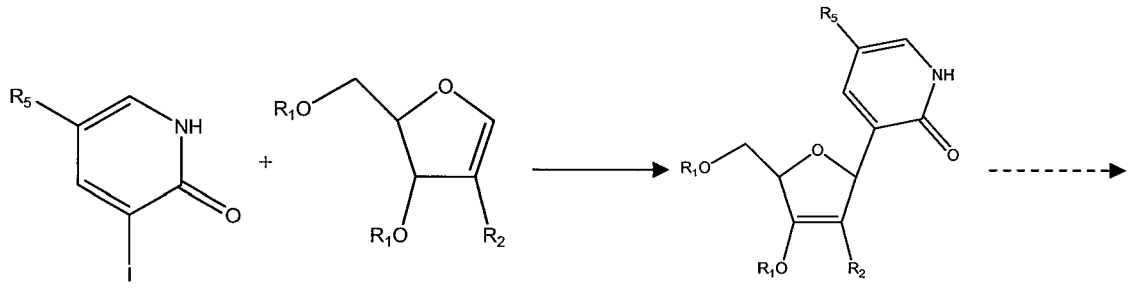
3) a fluorescent molecule selected from fluorescein, 6-carboxyfluorescein, tetramethyl-6-carboxyrhodamine, and derivatives thereof; and

4) biotin, dichloroacetyl group, fluorescein, 6-carboxyfluorescein, tetramethyl-6-carboxyrhodamine, or derivatives thereof introduced via a linker selected from an aminoalkyl group, an aminoalkenyl group and an aminoalkynyl group.

In the case of using unnatural base pairing s:y, misincorporation of U showed negligible selectivity, which indicated that s:y base pairing could be used for a new interaction between codon and anticodon when y was introduced at a specific site in mRNA. The present invention addresses site-selective introduction of a 5-substituted y derivative into RNA to generate RNA molecules having new functions. The inventive nucleoside or nucleotide having a 5-substituted pyridine base is advantageous in that it is less likely to cause unwanted base-base interference (*e.g.*, steric hindrance and unwanted binding observed during base pairing) when compared to a nucleoside or nucleotide having a substituent at the 1-, 2- or 6-position of the pyridine base.

Differences between the invention and the prior art

In contrast, Froehler *et al.* describe a method to synthesize nucleoside derivatives having pyridone with substituents, such as an alkyl group and alkynyl group at R5, wherein the method includes 3 steps comprising reactions which bind 5-iodo-2-pyridone to a ribose. In the method of Froehler *et al.*, the substituent at R3 is defined as –H or $-CH_3$. Therefore, the method is completely different from the method for synthesizing the nucleoside derivatives of the present invention having 2-pyridone (the position corresponding to R3 is keto group). Applicants provide the reaction scheme below for further clarity:



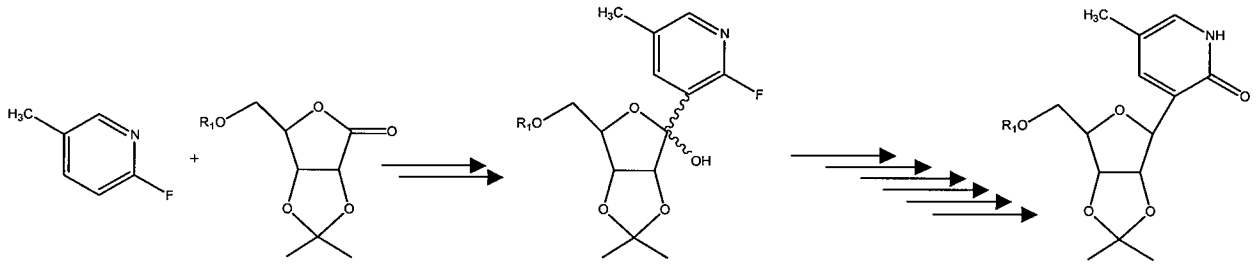
compound 1

compound 2

(the present invention)

Compound 2 of the above reaction having a halogen at the 5-position (R5) is one of the present nucleoside derivatives of the present invention. It is also an important intermediate for further synthesizing various 5-substiuted 2-pyridones. However, it is noteworthy that Compound 2 having a halogen at 5-position cannot be synthesized by the method disclosed in Froehler *et al.* This is because the yield of a radical at the 2-iodo position of the starting material, 2-halopyridone, is necessary to react with a ribose material via addition reaction. Thus, the halide compound 1 (R5 = halogen) cannot be used for the glycosidation reaction since the R5 position, as well as the 2-iodo position, would also react with the ribose material. Applicants also submit that it is also evident that Froehler *et al.* describe only simple substituents, such as an alkyl group or alkynyl group, but does not describe or suggest more complicated substituents, including compounds where R5 is halogen. There was no report before the present invention that the present nucleoside derivatives having various substituents at the 5-position of 2-pyridone have actually been synthesized by the method of Froehler *et al.*

With regards to Ohtsuki *et al.*, the invention is described as follows:



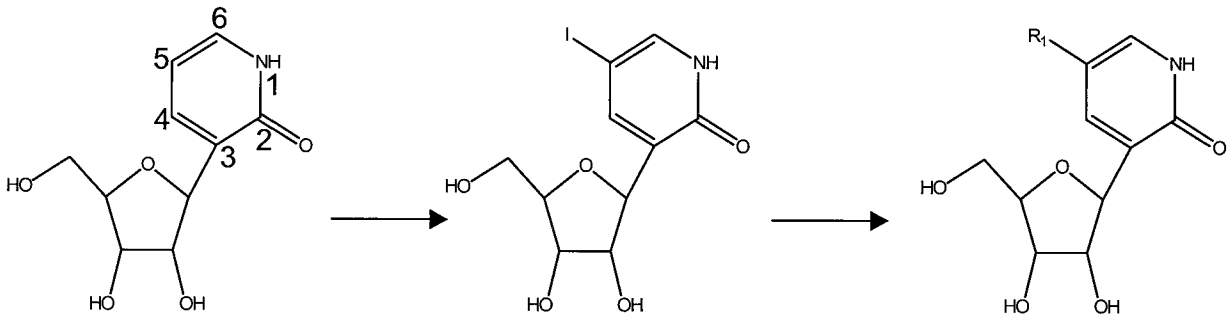
Ohtsuki *et al.* describe a method to synthesize the nucleoside derivatives of 5-methyl-2-pyridone. The method of Ohtsuki *et al.* comprises a step of binding 5-methyl-2-fluoropyridine with the ribose material. This is significantly distinct from the present method wherein the 5-position of 5-iodo-2-pyridine in the nucleotide derivatives is converted to a substituent selected from the group consisting of 1) to 4) described in Claim 2 of the present application. Ohtsuki *et al.* could be applicable only for 2-pyridone with only simple substituents, such as an alkyl group or alkynyl group at the 5-position. Therefore, the nucleoside derivatives of the present invention with complicated substituents cannot be synthesized by the methods described in Ohtsuki *et al.*

Unexpected Results with the Present Invention

As indicated in the provided Declaration, the present invention provides a nucleoside or nucleotide having a 5-substituted –2-oxo(1H)-pyridin-3-yl group as a base, wherein the 5-position of the base is substituted with a particular substituent selected from the group consisting of 1) to 4) described in Claim 2 of the present application. Applicants respectfully submit that the method of synthesizing the nucleoside or nucleotide of the present invention is novel. Ohtsuki *et al.*, and Froehler *et al.*, or combinations thereof, would not provide a method that would enable those skilled in the art to synthesize 5-substituted-2-pyridone derivatives of the

present invention. The nucleoside or nucleotide of the present invention is, therefore, novel, and is not easily obtained even by referring to the prior art documents. Applicants also submit that the nucleoside or nucleotide of the present invention first enabled those skilled in the art to introduce nucleosides having the 2-pyridone derivatives with various substituents at the 5-position into a specific position in DNA or RNA by replication or transcription mediated by artificial, extra base pair systems.

Applicants direct the Examiner's attention to the following reaction scheme to further clarify the novelty of the present invention.

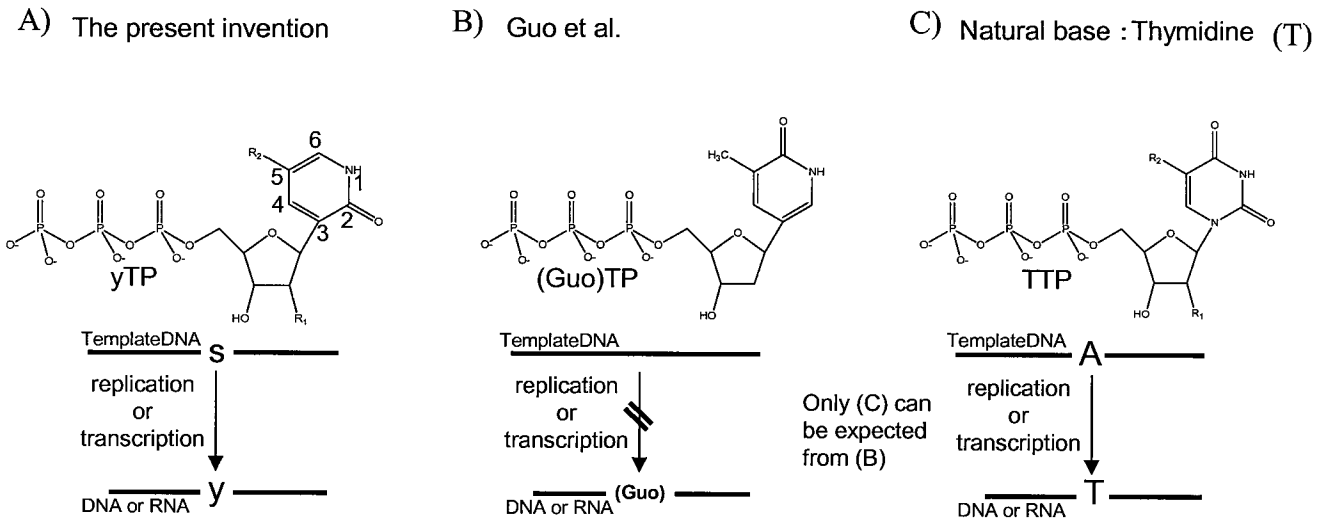


The method for synthesizing the nucleoside derivatives of 5-substituted-2-pyridone of the present invention comprises first synthesizing the nucleoside of 2-pyridone, iodinating the 5-position of the 2-pyridone moiety, and then introducing various substituents, preferably through linkers, such as alkyne, into the 5-position. Ohtsuki *et al.* and Froehler *et al.* do not disclose or suggest any method to enable synthesis of the various 5-substituted derivatives. The present specification provides for the first time a possible method to synthesize a wide variety of 2-pyridone derivatives with various useful substituents at the 5-position.

One of the technical points of the present method is to selectively iodinate the 5-position of 2-pyridone. One could expect that all of the 4-, 5- or 6-positions of the 2-pyridone moiety

might be iodinated. However, it has been demonstrated for the first time that only the 5-position is selectively reacted and iodinated. Accordingly, the nucleoside derivatives of the 5-substituted-2-pyridone of the present invention were obtained only after developing the synthesizing method disclosed in the present specification.

The following provides a comparison of the present invention to Froehler *et al.* and Ohtsuki *et al.*



s: 2-amino-6-thienylpurine
y: pyridine-2-one
x: 2-amino-6-(dimethylamino)purine

The nucleoside derivatives of the present invention can be incorporated into a specific position of nucleic acids by replication or transcription using a template DNA comprising an artificial base, such as "s" or "x", which specifically pairs with the 5-substituted pyridone (pyridine-2-one, y) in the polymerase reactions. The present invention is based on the following three technical ideas: (I) DNA or RNA polymerase can recognize and interact with the oxygen

atom at 2-position in the pyridone; (II) The nucleoside derivatives of the present invention can also form base pairs with an artificial base, such as such as "s" or "x" because the 6-position in the pyridone of the nucleoside derivatives has a small hydrogen atom to accommodate the complementary large artificial s or x base; (III) The nucleoside derivatives of the present invention wherein 2-pyridone thereof has various complicated substituents at the 5-position.

In contrast to the present invention, Guo *et al.*, (B), discloses an idea corresponding to (I), only, and does not provide any suggestion to achieve the present invention. In the first place, Guo *et al.* describes experiments using nucleoside derivatives wherein the keto group at 2-position is deleted from thymidine (T). Therefore, Guo *et al.* only provides, at most, a suggestion regarding relationships of the embodiment (B) to the embodiment (C) for the natural base pairs between A and T.

In addition, the present invention has identified that nucleoside derivatives having the 2-pyridone derivatives with various substituents at 5-position can be introduced into a specific position in DNA or RNA by replication of transcription mediated by artificial, extra base pairs. This is not possible with the teachings of either of Ohtsuki *et al.* or Froehler *et al.* The technical idea of (III) discussed above has first enabled application to replication or transcription using the specific artificial base pairs.

Applicants respectfully disagree that it would have been obvious to skilled artisan seeking alternative nucleobases to vary the positions of the substituents in the pyridine ring to produce another nucleobase to be used for the same purpose, namely for incorporation into nucleic acid molecules.

As described in the provided Declaration, Ohtsuki *et al.*, and Froehler *et al.*, or combinations thereof, would not provide a method that would enable those skilled in the art to synthesize 5-substituted-2-pyridone derivatives of the present invention.

In light of the above presently amended claims and remarks, because there is no disclosure, teaching, suggestion, reason or rationale provided in the Froehler *et al.* reference that would allow one of ordinary skill in the art to arrive at the instant invention as claimed, it follows that the same reference is incapable of rendering the instant invention obvious under the provisions of 35 USC § 103(a). Based upon the above, and applying the *Graham factors* analysis test, it is submitted that a *prima facie* case of obviousness has not been established.

Since the present invention is not obvious in light of Froehler *et al.*, the combinations of Froehler *et al.* and the above references also fail. The secondary references, Ohtsuki *et al.*, and Guo *et al.*, do not cure the deficiencies of Froehler *et al.* Therefore, the combinations of Froehler *et al.* with Ohtsuki *et al.*, and Guo *et al.*, do not arrive at the present invention. Based upon the above, and applying the *Graham factors* analysis test, it is submitted that a *prima facie* case of obviousness has not been established for any of the above mentioned claims. Applicants respectfully request reconsideration and subsequent withdrawal of the above rejections.

Issue Under 35 U.S.C. § 112, First Paragraph, Written Description

Claims 2-15 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. The Examiner asserts that the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed,

had possession of the claimed invention. This is a new matter rejection. The Examiner asserts that there is no support in the specification as filed for derivatives of dichloroacetyl group, fluorescein, 6-carboxyfluorescein, tetramethyl-6 carboxyrhodamine introduced at the 5- position of the nucleotide or nucleoside of the invention via a linker selected from an "aminoalkyl group, an aminoalkenyl group and an aminoalkynyl group." Applicants respectfully traverse.

Although Applicants disagree, in order to advance prosecution, claims 2, 3, 5, 11, and 13-15 have been amended, without prejudice or disclaimer, to further clarify and define the invention. Specifically, Applicants have amended the claims wherein the 5-position of the base is not substituted with a substituent that is an alkenyl group, an alkynyl group or an amino group.

In the Office Action (See page 6), the Examiner asserts that claims 2-15 do not meet the written description requirement for the following reason.

> *"Claim 2 and those claims dependent therefrom recite "biotin, dichloroacetyl group, fluorescein, 6-carboxyfluorescein, tetramethyl-6-carboxyrhodamine, or derivatives thereof introduced via a linker selected from an aminoalkyl group, an aminoalkenyl group and an aminoalkynyl group...It is noted that there is no support in the specification as filed for derivatives of dichloroacetyl group, fluorescein, 6-carboxyfluorescein, tetramethyl-6-carboxyrhodamine introduced at the 5-position of the nucleotide or nucleoside of the invention via a linker selected from an "aminoalkyl group, an aminoalkenyl group and an aminoalkynyl group."*

Applicants respectfully disagree. Applicants direct the Examiner's attention to the present specification descriptions at page 17, line 12, to page 19, line 8. The cited descriptions provide a detailed explanation regarding possible substituents at the 5-position. Further, Examples 11-15, as well as Figures 15-18, also disclose specific working examples wherein the 5-position substituent is biotin. By referring to the general but detailed explanations in the specification as well as working examples of biotin, Applicants submit that a skilled artisan can

easily understand that substituents listed in the claims can be easily introduced in the same manner as biotin.

Further, Applicants also point out that "Compound 10" in Figure 17 of the present application provides an embodiment wherein the dicholoroacetyl group is introduced via amino acetylenic linker.

Additionally, Applicants herein provide for the Examiner' consideration, Exhibit 1, which is supplemental data that was published soon after the present application (Exhibit 1: Kawai *et al.*, "*Site-Specific Fluorescent Labeling of RNA Molecules by Specific Transcription Using Unnatural Base Pairs*," J. Am. Chem. Soc., Vol. 127, pp. 17286-17295, (2005)). Exhibit 1 describes that 2-pyridone which has PAM (fluorescein), TAMRA, Dansyl at the 5-position have been synthesized by the method disclosed in the present specification. Exhibit 1 further describes that the synthesized 5-substituted 2-pyridone have been introduced into specific site(s) of RNA by transcription using a DNA template having the complementary artificial base. Therefore based on above, Applicants respectfully submit that the present specification provides sufficient disclosure in such a way to reasonably convey to the skilled artisan that the inventors had possession of the claimed invention.

Applicants respectfully request reconsideration and subsequent withdrawal of the above rejection.

CONCLUSION

A full and complete response has been made to all issues as cited in the Office Action. Applicants have taken substantial steps in efforts to advance prosecution of the present

application. Thus, Applicants respectfully request that a timely Notice of Allowance issue for the present case.

In view of the above remarks, it is believed that claims are allowable.

Should there be any outstanding matters within the present application that need to be resolved, the Examiner is respectfully requested to contact Paul D. Pyla, Reg. No. 59,228, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Dated: MAR 9 2009

Respectfully submitted,

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Attachments: 37 C.F.R. § 1.132 Declaration of Dr. Hirao

Exhibit 1: Kawai *et al.*, "*Site-Specific Fluorescent Labeling of RNA Molecules by Specific Transcription Using Unnatural Base Pairs*," J. Am. Chem. Soc., Vol. 127, pp. 17286-17295, (2005).

GMM/PDP/bpr